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REVIEW PAPER

The walls have ears: the role of plant CrRLK1Ls in sensing and transducing extracellular signals

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Abstract

In plants, organ formation and cell elongation require the constant adjustment of the dynamic and adaptable cell wall in response to environmental cues as well as internal regulators, such as light, mechanical stresses, pathogen attacks, phytohormones, and other signaling molecules. The molecular mechanisms that perceive these cues and translate them into cellular responses to maintain integrity and remodelling of the carbohydrate-rich cell wall for the coordination of cell growth are still poorly understood. In the last 3 years, the function of six membrane-localized receptor-like kinases (RLKs) belonging to the CrRLK1L family has been linked to the control of cell elongation in vegetative and reproductive development. Moreover, the presence of putative carbohydrate-binding domains in the extracellular domains of these CrRLK1Ls makes this receptor family an excellent candidate for coordinating cell growth, cell–cell communication, and constant cell wall remodelling during the plant life cycle.

Key words: *Arabidopsis*, cell wall, CrRLK1L, malectin-like domain, plant reproductive development, pollen tube reception, receptor-like kinase, signaling.

Introduction

Plant cells are surrounded by an extracellular matrix known as the cell wall. Cell walls are composed of a variety of carbohydrate polymers (including cellulose, hemicellulose, and pectins), and associated structural and regulatory proteins (Cosgrove, 2005). The rigid cell wall is responsible for resisting the high turgor pressure in plant cells and also provides protection against influences from the environment. Cell wall modifications are required for plant growth and development. The loosening of cell wall components determines the direction of growth and, as cells expand, new wall components must be synthesized in a coordinated manner. For example, during pollination, the pollen tube (PT) must sense signals from the surrounding transmitting tract of the pistil and coordinate cell wall modifications so that the sperm cells can be carried towards the ovules by polarized tip growth. When the PT reaches the ovary, it must change its direction of growth in order to enter the ovule and, finally, the PT must perceive a signal to alter its cell wall properties in order to cease its growth and rupture to release the sperm cells. Signaling between the cell wall and the inside of the cell is critical for proper development and the sensing of environmental cues, but little is known about the molecular mechanisms involved (Ringli, 2010; Seifert and Blaukopf, 2010). The plant-specific *Catharanthus roseus* RLK1-like (CrRLK1L) family, named after the first member which was identified from *C. roseus* cell cultures (Schulze-Muth *et al.*, 1996), has recently emerged as a candidate group for sensing changes at the cell wall and translating this information to cellular responses during both the reproductive and vegetative phases of the plant life cycle. The model plant *Arabidopsis thaliana* has 17 CrRLK1L family members (Hématy and Höfte, 2008). In this review, the functions of a subset of the CrRLK1L-encoding genes (Table 1) are explored and the significance of putative carbohydrate-binding domains in their extracellular domains is discussed.

Table 1. *The six characterized* Arabidopsis *CrRLK1Ls have been proposed to function in cell growth regulation*

References: a, Hématy *et al.*, 2007; b, Guo *et al.*, 2009*a*; c, Guo *et al.*, 2009*b*; d, Escobar-Restrepo *et al.*, 2007; e, Kessler *et al.*, 2010; f, Delauriers and Larsen, 2010; g, Miyazaki *et al.*, 2009; h, Boisson-Dernier *et al.*, 2009; i, Boisson-Dernier *et al.*, unpublished.

Name	AGI	Expression pattern	Subcellular localization	Kinase activity	KO or KD mutant phenotypes	OX phenotypes	Reference
THE1	At5g54380	• All vegetative tissues and more particularly in petioles, hypocotyls and root elongation zone • Up-regulated by BL, down-regulated in *bri1-5*	THE1–GFP, functional, uniform PM signal in hypocotyl epidermal cells	Yes	• *the1* no obvious phenotypes • *herk1 the1* strong stunted phenotypes • *the1* partially rescues *prc1-1*, *rsw1-10*, *eli1-1*, *pom1-2* hypocotyl growth defects	p*35S*::THE1–GFP • no phenotype in the WT • restores *prc1-1* phenotypes in *prc1-1 the1* background • enhances *eli1-1* stunted phenotype	a, b, c
FER	At3g51550	• All vegetative tissues, more particularly in petioles and hypocotyls • Strong in the synergids of the embryo sac • Up-regulated by BL or in *bes1-D*, down-regulated in *bri1-5*	FER–GFP, functional, uniform PM signal in leaf epidermis, but polarly localized towards the FA of synergids	Yes	• *fer* or *amiRNA-FER* lines very strong stunted phenotypes • etiolated *fer* seedlings hypersensitive to ethylene and insensitive to BL • *fer* plants resistant to powdery mildew • *fer* embryo sacs remain unfertilized due to PT overgrowth and absence of sperm release	ND	b, c, d, e, f
HERK1	At3g46290	• All vegetative tissues and more particularly in petioles and hypocotyls • Up-regulated by BL or in *bes1-D*, down-regulated in *bri1-5*	HERK1–GFP, functionality not demonstrated, uniform PM signal in hypocotyl epidermal cells	Yes	• *herk1* no obvious phenotypes • *herk1 thes1* strong stunted phenotypes	p*35Se-HERK1*::HERK1 • slightly increased petiole length in WT	b, c
HERK2	At1g30570	• Slightly up-regulated by BL	ND	ND	**herk2* no obvious phenotypes • *herk1 herk2 thes1* strong stunted phenotypes	ND	c
ANX1	At3g04690	• Preferential expression in pollen	ANX1/2–YFP, functional, polarly enriched in PM of the PT tip	ND	• single *anx1 or anx2* no obvious phenotypes	p*ACA9*::ANX1/2–YFP	g, h, i
ANX2	At5g28680				• *anx1 anx2* PTs burst prematurely before reaching the embryo sacs • *anx1 anx2* male sterile plants	• inhibits PT growth in WT • rescue *anx1 anx2* phenotypes	

BL, brassinolide; FA, filiform apparatus; KD, knock-down; KO, knock-out; ND, not determined; PM, plasma membrane; PT, pollen tube; OX, overexpression; 35S, CaMV 35S promoter; 35Se, CaMV 35S enhancers; WT, wild type.

CrRLK1Ls in reproductive development

FERONIA *in the female gametophyte*

During plant reproduction, the tip-growing PT grows through the female reproductive tissue to reach the female gametophyte (FG). Communication between the male and female occurs at several steps during reproduction (Dresselhaus, 2006). First, a pollen grain lands on a receptive stigma and hydrates, extending a PT that carries the two sperm cells towards the ovary. Next, the PT grows through the transmitting tract, absorbing nutrients from female tissues, from which the PT finally exits and is attracted to the FG.

Attracted by a chemotactic signal (Okuda *et al.*, 2009), the PT enters one of the synergids, stops growth, and bursts to release the two sperm cells to effect double fertilization. The CrRLK1L family member *FERONIA* (*FER*) plays a key role in PT reception, the final step of communication between the male and female gametophytes. In *fer* mutant FGs, the PT is attracted and penetrates the receptive synergid, but continues to grow and fails to rupture and release the sperm cells (Huck *et al.*, 2003), a phenotype also observed in *sirène* mutants, which are allelic to *fer* (Rotman *et al.*, 2003; Escobar-Restrepo *et al.*, 2007). A FER–green fluorescent protein (GFP) fusion protein that complements the *fer* mutation is localized to the plasma membrane in leaf epidermal cells, but, more importantly for its role in fertilization, FER–GFP is localized at the micropylar pole of synergid cells, a region known as the filiform apparatus (FA) (Escobar-Restrepo *et al.*, 2007). The FA is made up of a highly invaginated plasma membrane and a thickened cell wall, and constitutes the entry point of PTs into the FG (Higashiyama, 2002). FER could either act as receptor for a specific ligand from the PT, or could sense and respond to cell wall changes induced by the contact and entry of the PT into the synergid cell. Alternatively, it has been proposed that the *FER* pathway might be required for normal maturation of the synergids, allowing them to interact properly with PTs (Rotman *et al.*, 2008). However, this hypothesis does not explain why *fer*-like phenotypes are observed in some interspecific crosses (see below), where the synergids are wild type and fully mature.

Interspecific crosses between *A. thaliana* and *A. lyrata* or *Cardamine flexuosa* exhibit defects in PT reception similar to those observed in *fer* mutants (Escobar-Restrepo *et al.*, 2007). The extracellular domains of FER in these species exhibit high sequence variation that could indicate that FER may recognize species-specific ligands. Recently, three other mutants with *fer*-like phenotypes have been reported. *scylla* (unidentified; Rotman *et al.*, 2008), *lorelei* [*lre*; a putative glycosylphosphatidylinositol (GPI)-anchored protein; Capron *et al.*, 2008; Tsukamoto *et al.*, 2010], and *nortia* [*nta*; a mildew resistance locus o (MLO) family member; Kessler *et al.*, 2010] are all female gametophytic mutants that affect PT reception, exhibiting *fer*-like PT overgrowth inside the FG. An NTA–GFP fusion protein becomes polarly localized to the filiform apparatus at PT arrival, but this polar localization does not occur in *fer* mutant FGs, indicating that an active FER pathway required for sensing PT arrival is necessary for the redistribution of NTA–GFP (Kessler *et al.*, 2010). The mechanism through which FER, NTA, LRE, and other components cooperate in the synergid to control growth of the PT as it arrives at the FG remains elusive (Fig. 1). However, recent studies by two independent research groups have shed some new light on this intriguing male–female gametophytic communication by characterizing the functionally redundant CrRLK1Ls ANXUR1 (ANX1) and ANX2, which are required for normal PT growth (Boisson-Dernier *et al.*, 2009; Miyazaki *et al.*, 2009).

ANXUR1 *and* ANXUR2 *in the male gametophyte*

ANX1 and *ANX2* were analysed based on their preferential expression in pollen and/or their high sequence similarity to *FER*. Indeed, they are most closely related to *FER* in the *Arabidopsis* genome, and while *FER* is expressed everywhere in the plant except for pollen, *ANX1* and *ANX2* display the exact opposite expression pattern (Boisson-Dernier *et al.*, 2009; Miyazaki *et al.*, 2009). PTs carrying T-DNA insertions in both *ANX1* and *ANX2* are able to germinate but burst prematurely *in vitro* and *in vivo*, preventing them from reaching and fertilizing the FG (Boisson-Dernier *et al.*, 2009; Miyazaki *et al.*, 2009). Consequently, double homozygous *anx1 anx2* mutant plants were only rarely found in the progeny of selfed *anx1/anx1 anx2/ANX2*, or *anx1/ANX1 anx2/anx2* plants and were almost completely male sterile. Interestingly, ANX1–yellow fluorescent protein (YFP) and ANX2–YFP fusions are polarly localized in the plasma membrane of the PT tip (Boisson-Dernier *et al.*, 2009). Transforming *anx1/anx1 anx2/ANX2* plants with either an ANX1–YFP or an ANX2–YFP fusion under the control of a strong pollen promoter rescues the mutant phenotypes, indicating that these protein fusions are fully functional, while overexpressing them in wild-type PTs considerably slows PT growth (A. Boisson-Dernier and U. Grossniklaus, unpublished). Therefore, an appropriate threshold of functional ANX-RLK protein levels at the PT tip is required for PT growth, with an excess leading to growth inhibition while depletion impairs PT tip stability causing PT rupture.

The bursting of PTs *in vitro* is a common phenomenon as 2–10% of wild-type PTs rupture depending on the growth conditions. However, mutations that consistently lead to PT rupture are rare. Interestingly, *anx1 anx2* mutant PTs display the same phenotype as PTs lacking the cell wall-localized pectin methylesterase (PME; EC 3.1.1.11) encoded by *VANGUARD1* (*VGD1*; Jiang *et al.*, 2005). PMEs catalyse the specific demethylesterification of the linear homopolymer (1,4)-linked-α-D-galacturonic acid homogalaturonan (HGA), a major pectic constituent of the cell wall (Pelloux *et al.*, 2007). HGA is deposited in the apoplast through the secretory pathway in a predominantly methylesterified state (Mohnen, 2008). HGA is subsequently demethylesterified within the cell wall by PMEs, the activities of which are regulated by the cell wall's pH, PME inhibitor (PMEI) proteins, or even intramolecularly by PMEI-like domains (Bosch *et al.*, 2005; Juge, 2006; Rockel *et al.*, 2008). Demethylesterified pectins are able to form Ca^{2+} bonds inducing gel formation that rigidifies the cell wall. Moreover, they are more accessible to degradation by pectinolytic enzymes, which affect cell wall composition and lead to the production of signaling molecules called oligogalacturonides. Therefore, the balance between methyl-esterified 'loose' and demethylesterified 'rigid' forms of HGA is essential for wall mechanics and must be tightly regulated during cell growth and development (Ridley *et al.*, 2001; Willats *et al.*, 2001; Pelloux *et al.*, 2007; Hématy *et al.*, 2009; Wolf *et al.*, 2009). Thus, it is not surprising that many studies indicate a central role for asymmetrically distributed

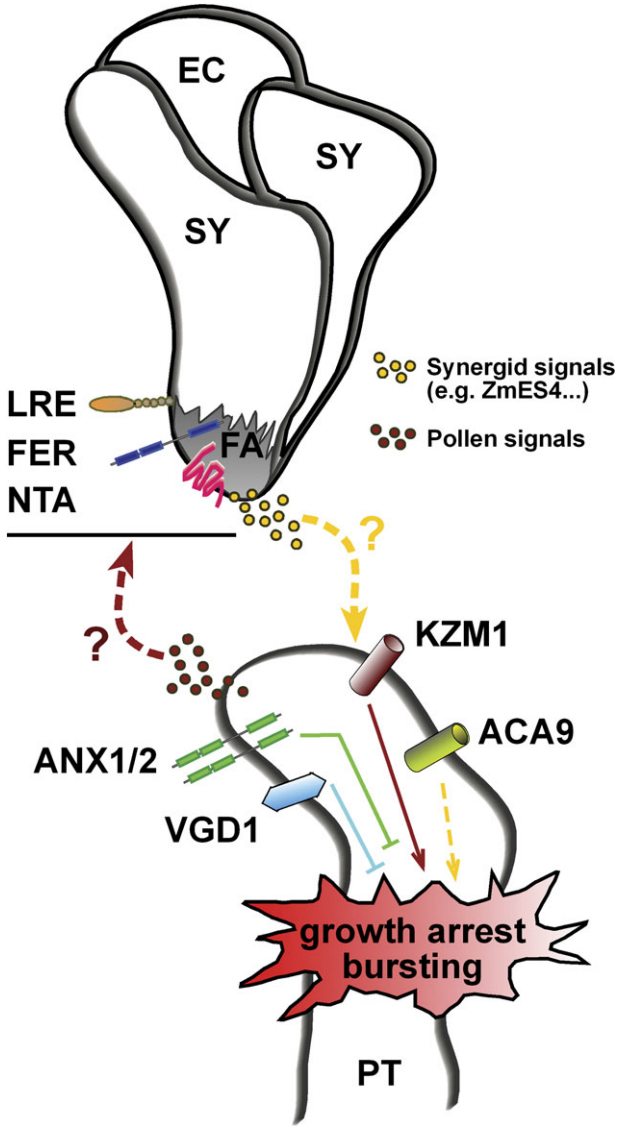

Fig. 1. Molecular components that play a role in the male–female gametophytic dialogue during pollen tube reception. When the PT reaches the vicinity or comes into contact with the filiform apparatus of the receptive synergid, unknown pollen molecules and/or physical interactions between the two gametophytes are thought to signal the female gamete to prepare itself for penetration and fertilization through the activation of a female signaling module composed of the FA-localized FER-RLK, the putative GPI-anchored protein LRE, and the seven-transmembrane domain MLO protein, NTA. How these female components cooperate remains unclear, but FER activity is required to relocalize NTA to the membrane-enriched FA, the entry point for the PT. Subsequently, the FG signals back to its male partner so that it stops growth and ruptures to deliver the two sperm. This is achieved via synergid signals, such as the cysteine-rich small protein ZmES4, which triggers PT rupture possibly through an osmotic shock due to the opening of the PT-localized K^+-channel KZM1 and subsequent K^+ influx. Other male gametophytic components, such as the tip-polarized plasma membrane-localized ANX-RLKs or the cell wall-localized pectin methylesterase VGD1, promote PT growth within the female sporophytic tissues in order to prevent PT rupture before reaching the FG. However, unlike KZM1, it is still unclear whether the FG, via unknown synergid signals, is able to deactivate these male components to trigger PT growth arrest and sperm cell discharge. Finally, the PT-localized Ca^{2+} channel ACA9 appears to have a complex role by promoting both PT growth and sperm cell discharge. EC, egg cell; SY, synergids.

pectins, as well as PMEs and PMEIs during PT growth (Chebli and Geitmann, 2007; Cheung and Wu, 2008; Zonia and Munnik, 2009; Wolf *et al.*, 2009).

Although the similar phenotypes of *anx1 anx2* and *vgd1* PTs could be merely circumstantial, the facts that (i) these mutant PTs consistently burst prematurely; (ii) ANX-RLKs and pollen-expressed PMEs/PMEIs are in close proximity to each other at the PT tip; and (iii) the synthesis, distribution, and degree of esterification of pectins play a major role in PT growth, indicate that ANX-RLKs and PMEs/PMEIs may cooperate during PT growth (Fig. 1). For example, the ANX-RLKs could regulate the activity or the distribution of PMEs/PMEIs in PTs in order to readjust the balance of methylesterified and demethylesterified HGAs. Alternatively, HGAs might bind the extracellular domains of ANX-RLKs to regulate their activity and,

therefore, modulate the corresponding signaling cascade. In this respect, it is noteworthy that the extracellular regions of the RLKs WALL-ASSOCIATED KINASE1 (WAK1) and WAK2 have been shown to bind demethylesterified HGAs and that HGAs can regulate WAK2 activity (Kohorn *et al.*, 2009). However, the extracellular regions of these RLKs share little similarity to those of CrRLK1Ls and do not have malectin-like domains (see below). The study of genetic and molecular interactions, as well as analyses of the cell wall composition in these mutant PTs will shed more light onto interactions of the ANX-RLKs and pectin esterification.

The *anx1 anx2* double mutant phenotype indicates that ANX-RLKs act redundantly to maintain PT integrity during its growth through the female sporophytic tissues. However, whether ANX-RLKs are also involved in the male–female gametophytic dialogue that controls PT reception remains an open question. To date, there is no clear evidence to demonstrate such a role. However, it is suggested by the fact that (i) the premature rupture of *anx1 anx2* PTs shares characteristics with the explosive PT discharge in the receptive synergid (Higashiyama *et al.*, 2000; Rotman *et al.*, 2003; Palanivelu and Preuss, 2006; Sandaklie-Nikolova *et al.*, 2007); (ii) ANX-RLKs are the male counterpart of FER, enabling the FG to control PT reception; and (iii) FER and ANX-RLKs are localized to the FA and the PT tip, the regions of male–female gametophytic contact during PT reception, respectively. Therefore, disrupting the *ANX*-dependent pathway at the tip of the PT that grows into the FA would enable the FG to trigger PT growth arrest and sperm discharge efficiently. For example, it is possible that cell wall-modifying enzymes such as polygalactorunases, expansins, PMEs, PMEIs, or small defensin-like proteins, which are enriched in the synergids' FA and possibly under the control of the *FER*-dependent pathway, could inhibit the *ANX*-dependent pathway leading to PT rupture (Dresselhaus, 2006; Jones-Rhoades *et al.*, 2007; Okuda *et al.*, 2009; Punwani *et al.*, 2009; Amien *et al.*, 2010; Wuest *et al.*, 2010). Indeed, Amien and colleagues recently reported that the synergid-expressed defensin-like ZmES4 (*Zea mays* Embryo Sac 4) protein is able *in vitro* to rapidly trigger maize PT membrane depolarization and rupture in a species-preferential manner (Amien *et al.*, 2010). Moreover, ZmES4 is able to induce the opening of the pollen-expressed potassium channel KZM1 (K^+-channel *Zea mays* 1) in a heterologous system, showing for the first time that a male component can be the target of a female signaling molecule (Amien *et al.*, 2010; Fig. 1). However, direct evidence for the involvement of KZM1 in PT growth arrest and sperm cell discharge *in planta* is still awaited. Previously, the Ca^{2+} channel ACA9 has also been reported to be required for PT discharge, as half of the *aca9* mutant PTs appear unable to rupture once they reach the FG (Schiøtt *et al.*, 2004). However, unlike the female components FER, LRE, NTA, and ZmES4, ACA9 does not inhibit PT growth but rather promotes it, as *aca9* PTs grow much more slowly than the wild type both *in vitro* and *in vivo* (Schiøtt *et al.*, 2004; Fig. 1).

CrRLK1Ls and vegetative development

Recently, several members of the CrRLK1L family of RLKs have also been shown to be involved in controlling cell growth during different stages of vegetative development. Plant steroid hormones known as brassinosteroids (BRs) regulate cell elongation as well as many other developmental processes (Belkhadir and Chory, 2006). BR treatment induces cell elongation, while mutations in BR signaling components such as BRI1 (BRASSINOSTEROID INSENSITIVE 1), the BR receptor, cause dwarf phenotypes. The CrRLK1L receptor kinases HERCULES RECEPTOR KINASE1 (HERK1), HERK2, THESEUS1 (THE1), and FER were all identified by microarray analysis as genes whose expression is induced by the BR pathway. Homozygous *feronia* mutant plants (Kessler *et al.*, 2010), transgenic plants with induced down-regulation of *FER* by an artificial microRNA (amiRNA) (Guo *et al.*, 2009*a*), double mutants between *herk1* and *the1*, and *herk1 herk2 the1* triple mutants (Guo *et al.*, 2009*b*) all have stunted growth phenotypes with shorter petioles and hypocotyl cells than the wild type.

The interaction between *CrRLK1L* genes and the BR pathway was investigated to determine if the genes are acting in the same or in parallel pathways (Guo *et al.*, 2009*a*). Triple mutants between *herk1 the1* and the weak *bri1-5* loss-of-function allele have an enhanced *bri1* dwarf phenotype, while the combination of *herk1 the1* with *bes1-D*, a gain-of-function mutant with constitutive BR responses including excessive cell elongation, showed partial suppression of the cell elongation phenotype. This genetic analysis indicated that HERK1 and THE1 cooperate with the BR pathway to regulate cell elongation. Likewise, homozygous *fer-2* loss-of-function mutants have recently been shown to modulate the BR response in a light-dependent manner (Deslauriers and Larsen, 2010). Hypocotyls of light-grown *fer-2* seedlings showed an enhanced BR response, while dark-grown hypocotyls were partially BR insensitive and displayed an enhanced ethylene response.

Cell elongation requires modifications of the plant cell wall (Darley *et al.*, 2001). BR-induced cell elongation is thought to occur through the up-regulation of cell wall-loosening enzymes (Becnel *et al.*, 2006; Palusa *et al.*, 2007 Darley *et al.*, 2001). Consistent with a role for *HERK1* and *THE1* in cell elongation, the transcription of cell wall-loosening enzymes, including expansin, pectin lyase-like, and xyloglucan endotransglycosylase/hydrolase genes, is down-regulated in a *herk1 the1* double mutant (Guo *et al.*, 2009*a*). However, the function of CrRLK1Ls is not simply to modify cell walls and induce cell elongation. The *the1* mutant was originally identified as a suppressor of *procuste1* (*prc1*), a mutation in the cellulose synthase gene *CESA6* (Hématy *et al.*, 2007). The *prc1* mutants are cellulose deficient and have short hypocotyls when grown in the dark, while *prc1 the1* double mutants have hypocotyl lengths intermediate between the wild type and *prc1,* but the same cellulose deficiency as *prc1* mutants. The short hypocotyls and dwarf phenotypes of two other cellulose-deficient mutants,

cesA3$^{eli1\text{-}1}$ and *pompom1*, are also partially suppressed by *the1*. Moreover, overexpression of *THE1* in these mutant backgrounds enhanced their dwarf phenotype, indicating that, in cellulose-deficient backgrounds, *THE1* actually suppresses growth. However, in a wild-type background, overexpression of *THE1* has no apparent effect on vegetative growth.

The seemingly opposite phenotypic effects of *the1* in a cellulose-deficient background and in a double mutant background with *herk1* indicate that the function of these RLKs is probably dependent on the cell wall context. When cellulose levels are lower than normal in the *cesA* mutant background, *THE1* senses the abnormal or weak cell wall and inhibits growth. However, if the cell wall is intact, *THE1* (along with *HERK1* and/or *FER*) could participate in cell wall loosening to allow BR (and probably other pathways) to direct cell elongation.

A cell wall-sensing role for the CrRLK1L family members is further supported by the recent discovery of a function for *FER* in powdery mildew susceptibility (Kessler *et al.*, 2010). The link between *FER* and fungal invasion arose from the identification of NTA as an MLO protein family member. *MLO* genes were first identified as powdery mildew susceptibility genes in barley (Buschges *et al.*, 1997), with mutations in some *MLO* genes leading to powdery mildew resistance in barley, *Arabidopsis*, and tomato (Piffanelli *et al.*, 2004; Consonni *et al.*, 2006; Bai *et al.*, 2008). Since *fer* and *nta* mutants displayed a similar PT reception phenotype (Kessler *et al.*, 2010) and, unlike *NTA*, *FER* is also expressed in leaf epidermal cells, *fer* mutants were examined for powdery mildew resistance. Interestingly, homozygous *fer* mutants showed a similar powdery mildew resistance phenotype to that seen in *mlo* mutants. These results indicate that FER may act as a sensor of cell wall alterations due to contact with tip-growing cells and may coordinate the function of distinct MLO proteins in a tissue-dependent manner to allow entry of fungal hyphae and PTs, respectively.

Putative ligands of the CrRLK1L family

The huge family of RLKs in plants is classified into 45 subfamilies that can be differentiated according to sequence similarity of their extracellular domains and their domain organization (Shiu and Bleecker, 2003). Although the presence of known domains in the extracellular half of RLKs can be used in some cases to predict the nature of the corresponding ligands (peptides, proteins, hormones, carbohydrates, etc.), so far only a few ligand–RLK interactions have been biochemically characterized (Butenko *et al.*, 2009). The extracellular domain sequence for all CrRLK1Ls was recently submitted to a Pfam 24.0 analysis (http://pfam.sanger.ac.uk/; Finn *et al.*, 2010) to search for similarities to domains of known function. Surprisingly, this analysis showed that most of the CrRLK1Ls contain two regions with similarity to the carbohydrate-binding domain of a newly characterized *Xenopus laevis* protein named malectin (Schallus *et al.*, 2008; Fig. 2).

The *Malectin* gene is widely expressed throughout *Xenopus* development and encodes an endoplasmic reticulum (ER)-localized protein containing an N-terminal signal peptide (SP), followed by a globular domain and a C-terminal transmembrane helix (TM). The function of malectin has not been elucidated, but its globular domain shares a close structural similarity to carbohydrate-binding modules from prokaryotes. In addition, nuclear magnetic resonance-based screening demonstrated that malectin binds maltose and related oligosaccharides, while analyses with carbohydrate microarrays containing various mammalian glycans identified di-glucose-*N*-glycan (Glc_2-*N*-glycan) as the preferential binding partner. Together, these data led the authors to suggest a role for malectin during the control of *N*-glycosylation in the ER (Schallus *et al.*, 2008). The maltose-binding domain, hereafter referred to as the malectin domain (Pfam PF11721), is well conserved in animals (Schallus *et al.*, 2008). In plants, although more divergent, malectin-like domains are found frequently in the extracellular halves of RLKs and in many other proteins with various domain combinations. Interestingly, the association of an extracellular malectin-like domain with an intracellular kinase seems to be unique to the plant kingdom. The plant malectin-like domain with highest similarity to malectin is found in the extracellular part of the leucine-rich repeat LRR-RLK At1g53430, where identity and similarity reach 21% and 35% over 160 amino acids, respectively (Fig. 2B). According to Pfam analysis, 10 CrRLK1L members have two significant malectin-like domains, three have one significant and one non-significant malectin-like domain, while only two members contain two non-significant malectin-like domains (Fig. 2A). The domain with highest similarity to malectin in the CrRLK1L family is the malectin-like domain adjacent to the TM of ANX2 with identity and similarity scores of 19% and 36%, respectively (Fig. 2). It is noteworthy that the tandem domain organization does not correspond to a duplication of the same malectin-like domain, i.e. the conservation between two domains at the same position (adjacent to the SP or the TM) for different CrRLK1L proteins can reach as high as ~90% (e.g. ANX1 and ANX2), while, within the same protein, identity between the two malectin-like domains is very low (e.g. ~10% for the two domains of ANX1).

Considering (i) the low homology between *X. laevis* malectin and the CrRLK1L malectin-like domains; (ii) the weak conservation in CrRLK1L malectin-like domains of the residues that mediate the malectin–Glc_2 interaction; and (iii) the unique tandem-domain organization in the CrRLK1L members and their plasma membrane localization, it is unlikely that the CrRLK1L family members play a similar role to the one proposed for *X. laevis* malectin in the quality control of *N*-glycosylated proteins in the ER. However, it is likely that the extracellular part of the CrRLK1L members mediates binding to carbohydrates. It is too early to predict whether carbohydrates that may be

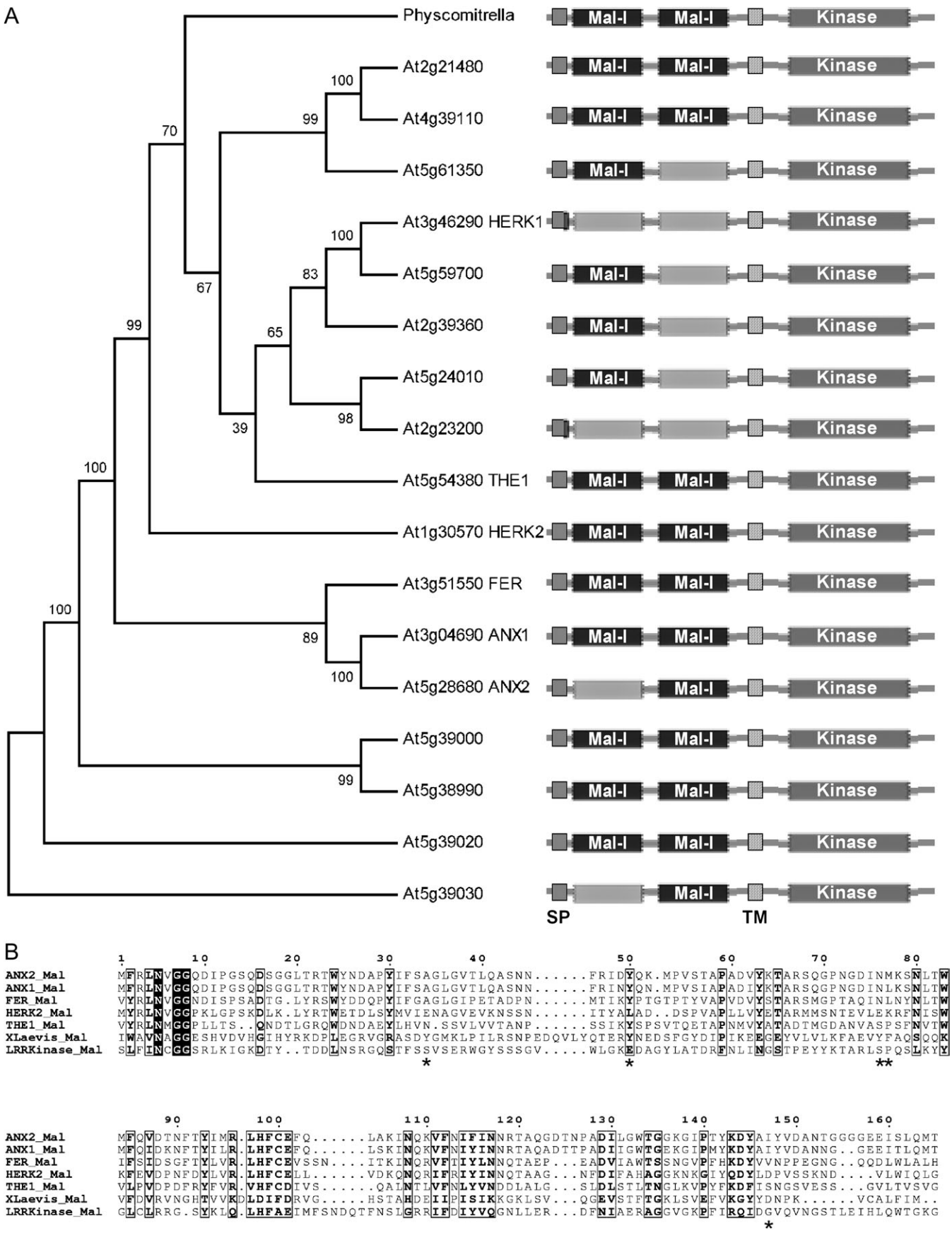

Fig. 2. Phylogenetic tree of the 17 members of the *Arabidopsis thaliana* CrRLK1L family with their domain organization and alignment of the CrRLK1L malectin-like domains. (A) Multiple alignments were performed with ClustalW 1.83 and the phylogenetic tree was

bound by CrRLK1L extracellular domains are oligo- or polysaccharides from cell wall constituents, by-products of cell wall degradation, or membrane-associated or secreted glycosylated proteins. However, development of a carbohydrate microarray binding assay that would include plant oligosacharrides, glycosylated proteins, and cell wall degradation products could allow the identification of the preferential binding partners for the CrRLK1L members similarly to what was reported for the *X. laevis* malectin (Schallus *et al.*, 2008).

Partial functional redundancy among CrRLK1L family members

Expression analyses of CrRLK1L family members indicate that some display overlapping expression patterns in vegetative tissues, some in pollen, while others are similarly induced by stresses and hormones, suggesting the existence of functional redundancy between some members (Guo *et al.*, 2009*a*; Hématy and Höfte, 2008). Indeed, functional redundancy has been reported in pollen for *ANX1* and *ANX2*, which is not surprising as their extracellular domains share 83.2% identity at the amino acid level, suggesting that ANX1 and ANX2 could bind the same ligand (Boisson-Dernier *et al.*, 2009; Miyazaki *et al.*, 2009). However, the partial redundancy observed between *THE1*, *HERK1*, and *HERK2* during vegetative growth (Guo *et al.*, 2009*a*, *b*) is more intriguing as they belong to different subclasses and their extracellular domains share more limited identity (38.7% for HERK1–THE1, 30.3% for THE1–HERK2, and 28.2% for HERK1–HERK2; Fig. 2A). Moreover, because FER-RNAi (RNA interference) lines display the same severe stunted growth phenotype as the triple *herk1 herk2 the1* mutant (Guo *et al.*, 2009*a*, *b*), Guo and colleagues hypothesized that in vegetative tissues CrRLK1Ls could act cooperatively through heterodimerization, with FER acting as a co-receptor for HERK1, HERK2, and THE1. Alternatively, considering the divergence in the extracellular domains of these CrRLK1Ls and the compensatory mechanisms that occur in cell wall remodelling, it is proposed that each CrRLK1L may bind a specific cell wall carbohydrate or glycoprotein to monitor cell wall integrity and that loss of this monitoring for some constituents can be partially compensated by other CrRLK1L-dependent pathways, while loss of the FER-dependent monitoring cannot. Cell wall components such as the rhamnogalacturonan II (RG-II) pectin or glycosylated proteins can carry very complex and diverse carbohydrate decorations. Thus, in another model, CrRLK1Ls could bind different sugar decorations of the same complex cell wall constituent and the lack of recognition could have more drastic consequences for some decorations than that for others.

Conclusion

Since the characterization of THE1 and FER, the first *Arabidopsis* CrRLK1L members, 3 years ago, the hypothesis that the CrRLK1L family could function in the control of cell wall integrity becomes more plausible with each new CrRLK1L-related study. Simultaneously, the complexity of such a cell wall integrity sensing system is becoming more and more apparent. In Fig. 3, a model for the function(s) of the CrRLK1Ls is presented. During normal developmental processes, these RLKs could be responsible for monitoring cell wall integrity, probably in terms of molecular composition, in order to fine-tune the responses from other signaling pathways such as those of phytohormones. Alternatively, CrRLK1Ls could specifically recognize carbohydrate ligands produced in response to environmental

reconstructed with MEGA4 using the protein sequence parsimony method. The percentages of replicate trees in which the associated sequences clustered together in the bootstrap test (1000 replicates) are shown next to the branches. A CrRLK1L homologue in *Physcomitrella patens* (XP_001760700) was used as an outgroup. The domain organization for each CrRLK1L member is drawn on the right. The extracellular domain sequence alone (first 430 amino acids) for each member was submitted on 7 April 2010 to PFAM sequence analysis (http://pfam.sanger.ac.uk/). Nine out of 17 CrRLK1L members carry two malectin-like domains [significant PFAM match with the malectin core domain (Schallus *et al.*, 2008)] in their extracellular part, six display one malectin-like domain paired with a more divergent domain (Pfam match for malectin domain but with insignificant score), and two members (HERK1 and At2g23200) exhibit a pair of divergent domains. Protein sequences were obtained from the PFAM-compatible ProteinKnowledgebase (UniProtKB, http://www.uniprot.org/) and are Q9SCZ4 (At3g51550, FER), Q9LX66 (At3g46290, HERK1), Q9SA72 (At1g30570, HERK2), Q9LK35 (At5g54380, THE1), Q9SR05 (At3g04690, ANX1), Q3E8W4 (At5g28680, ANX2), Q9T020 (At4g39110), Q9SJT0 (At2g21480), O80623 (At2g39360), Q9FID8 (At5g39000), Q9FID6 (At5g39020), Q9FN92 (At5g59700), Q9FLJ8 (At5g61350), Q9FID9 (At5g38990), Q9FID5 (At5g39030), Q9FLW0 (At5g24010), and O22187 (At2g23200). Black boxes are malectin-like domains with significant match to the malectin domain according to Pfam analysis. Unlabelled grey boxes represent more divergent domains, that is to say regions that match with the malectin domain but with an insignificant score according to Pfam analysis. (B) Alignment of the malectin-like domains adjacent to the transmembrane domain for THE1, HERK2, FER, ANX1, and ANX2 with the malectin core domain of the malectin protein from *Xenopus laevis* (Q6INX3) was performed with ClustalW 1.83 and its presentation with ESPript2.2 (http://espript.ibcp.fr/ESPript/ESPript/). The malectin-like domain of LRR-RLK At1g53430 (C0LGG8) was included. The aromatic residues and the aspartate, which in *X. laevis* malectin mediate interactions with the glucose residues (Schallus *et al.*, 2008), are marked by an asterisk and do not appear to be conserved in plant RLKs.

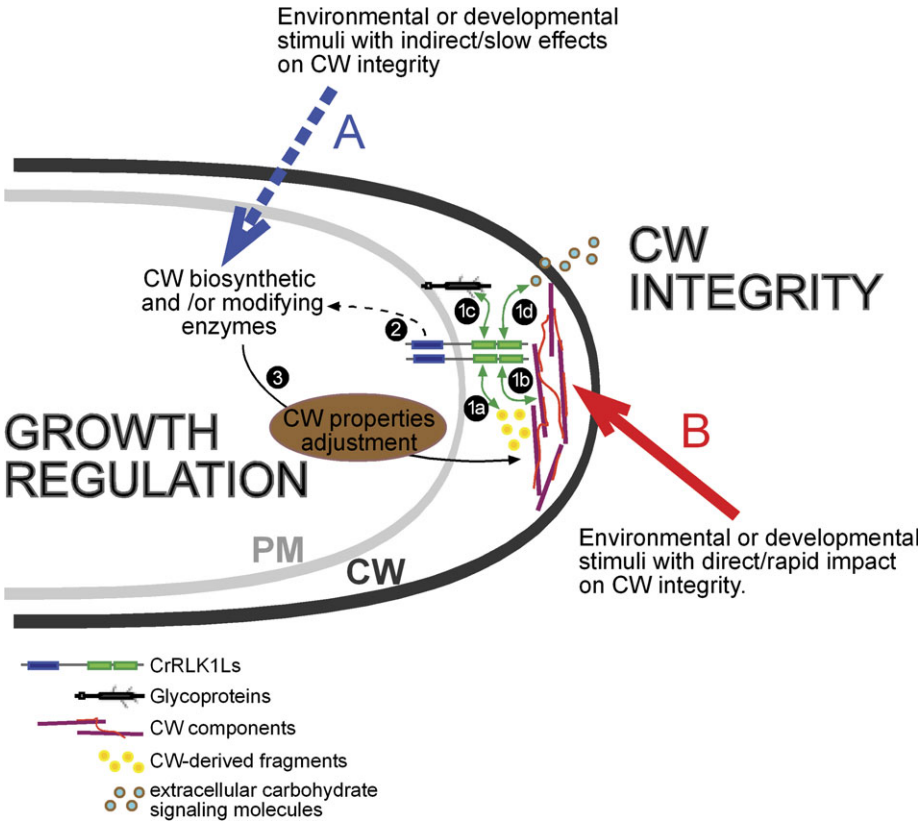

Fig. 3. A putative model for CrRLK1Ls signaling pathways in the control of cell wall integrity and growth regulation. Via their malectin-like extracellular domains, CrRLK1Ls could bind carbohydrate ligands derived from the degradation of cell wall components (1a), carbohydrate-rich cell wall components (1b), glycoproteins at the plasma membrane (1c), or secreted carbohydrate-rich signaling molecules derived from neighbouring cells (1d). This information is processed in the cytoplasm and relayed to the apoplast for direct or indirect regulation of (2) synthesis, secretion, or activity of cell wall biosynthetic and/or modifying enzymes in order to adjust cell wall composition for proper growth or the appropriate response to signaling molecules from the cellular environment (3). On one hand, the surveillance via CrRLK1Ls could constantly occur to fine-tune 'indirect' cell wall changes induced by environmental or developmental cues such as brassinosteroids and other signaling molecules (A). On the other hand, CrRLK1Ls could spontaneously sense cell wall alterations triggered directly by environmental or developmental cues to adjust cell wall properties (B).

cues or developmental processes that have rapid effects on cell wall composition and integrity, such as mechanical stress, fungal invasion, or male–female gametophyte interactions. The next challenge in the elucidation of CrRLK1L function is to identify not only some specific ligands but also other signaling components involved in CrRLK1L-mediated control of cell wall regulation and integrity. Regulators of secretion, distribution, or regulation of cell wall biosynthetic or modifying enzymes are good candidates for downstream effectors of CrRLK1L signaling pathways. Moreover, the potential binding of the tandem malectin-like domains of CrRLK1Ls to carbohydrates opens up the possibility that the variation in the CrRLK1L extracellular domains (both within the family and between species) could have evolved to reflect both tissue-dependent and species-dependent variations in the composition of cell wall components or glycosylated proteins (Knox, 2008; Sarkar *et al.*, 2009).

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